#223

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-Second Street New York 17, N.Y.

Cf. #128
Activated 7/1/56
Renewed 7/1/57
Renewed 7/1/58

Application for Research Grant

Date: December 7, 1958

1. Name of Investigator: 1. Paul S. Larson, 2. H.B. Haag, 3. Herbert McKennis, Jr.

2. Title: 1. Professor of Pharmacology, 2. Professor of Pharmacology, 3. Professor of Pharmacology

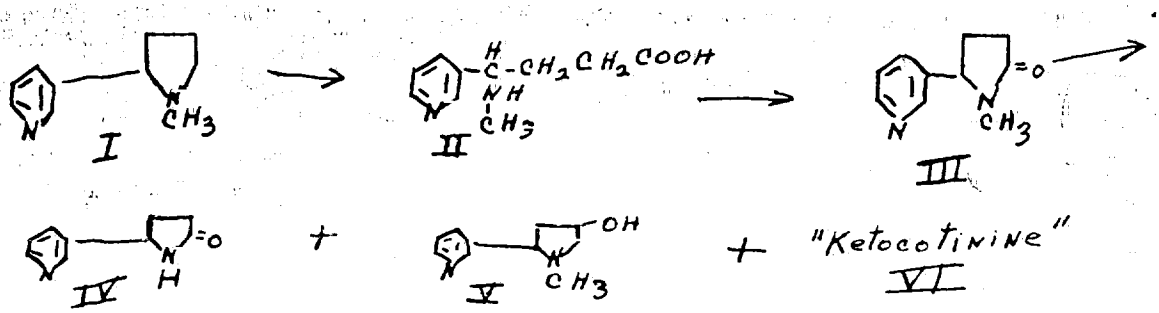
3. Institution & Address: Medical College of Virginia, Richmond 19, Virginia

4. Project or Subject: Enzymatic Transformations of Nicotine and Related Compounds

(Continuance application for period of July 1, 1959 - June 30, 1962.)

5. Detailed Plan of Procedure (use reverse side if additional space is needed):

During the past two and one-half years procedures for the isolation and synthesis of a variety of nicotine metabolites have been developed. In summary the following relationships have been established:



The foregoing compounds represent approximately 30% of the total metabolites of nicotine in the dog. y-3-Pyridyl-y-methylaminobutyric acid (II), cotinine (III), desmethylcotinine (IV) and hydroxycotinine (V) have all been isolated in pure condition and identified by comparison with synthetic compounds. With the exception of cotinine these are all new organic compounds for which synthetic methods were developed. These compounds have now been prepared in sufficient quantity to permit physiological studies. The syntheses provide also new total synthetic routes to both nornicotine and nicotine (I) and will allow investigators to selectively label nicotine, nornicotine or metabolites with isotopes for further study. Current evidence points to the occurrence of at least 15 Koenig positive metabolites

of nicotine. An even larger number of Koenig negative metabolites may be revealed by use of procedures which have been developed to isolate the Koenig positive materials. Preliminary physiological studies with the isolated metabolites indicates that pressor activity is significantly or totally obliterated by the known biotransformation. y-3-Pyridyl-y-methylaminobutyric acid may, however, have an antidiuretic activity greater than that of nicotine (without the side effects). Nicotine metabolism in the human has been found to parallel (by chromatograms of the chloroform soluble fractions) that of the dog. Smokers excrete, however, Koenig positive material which may arise from non-nicotine pyridine components of smoke (see separate application).

It is proposed to study further nicotine metabolism, separate the metabolites chromatographically, identify the metabolites, and make possible physiological studies of the metabolites. Attention will be given to the possible reversible nature of reactions leading in vivo to the lactam metabolites, desmethylcotinine, hydroxycotinine, and cotinine. The possibility of relating nicotine intake to specific metabolites in the urine will also be considered. Two and one-half years of TIRC supported work has given rise to the publications given on the attached sheet.

6. Budget Plan:

Social Security	$ 240
Salaries	$17,900
Expendable Supplies	$ 4,500
Permanent Equipment	$ 6,500
Overhead (10%)	$ 3,003
Other Travel	$ 890
Total	$33,033

7. Anticipated Duration of Work: Three years (renewal of present three-year period which expires June 30, 1959). Progress will be reported at intervals and in the literature according to custom and past practice.

8. Facilities and Staff Available: General pharmacologic and biochemical equipment, Lardy type Warburg apparatus, refrigerator centrifuge, C^{14} counting equipment, nuclear instrument gas flow counter with automatic sample changer, paper chromatographic equipment, polarimeter, greenhouse facilities for growing C^{14} labelled plants, high pressure hydrogenation apparatus. Full time staff: Lennox B. Turnbull, Ph.D., M.S. to be hired. Part time includes: Drs. Herbert McKennis, Jr., Paul S. Larson, H.B. Haag, and E.R. Bowman, Public Health Research Fellow.

9. Additional Requirements: What, if any, will depend upon how the project develops.

10. Additional information (including relation of work to other projects and other sources of supply): The American Tobacco Company has provided C^{14} labelled nicotine for metabolic studies. E.R. Bowman has received a Public Health Research Fellowship from the National Heart Institute which will make possible his part-time participation in the project. He previously devoted full time and has already begun to train new workers in the necessary techniques.

The breakdown for salaries is as follows:

Lennox B. Turnbull, Ph.D.		$ 8,500
To be filled	M.S.	$ 6,200
Technician part time	B.S.	$ 2,200
Animal man part time		$ 1,000
		$17,900

The breakdown on permanent equipment is as follows:

Gas chromatographic equipment	$ 4,200
X-Y recorder for autotitrator	$ 1,850
Partial cost of autotitrator	$ 450
	$ 6,500

Signature /s./ Paul S. Larson
Director of Project

/s./ B. B. Smith
Business Officer of the Institution

Publications (TIRC supported project work):

1) Synthesis and properties of pyridylalanines, Herbert McKennis, Jr. and Edward R. Bowman, The Virginia Journal of Science, 8, 314 (1957).

2) Metabolism of y-(3-pyridyl)-y-oxobutyric acid, Lennox B. Turnbull, Edward R. Bowman and Herbert McKennis, Jr., Federation Proceedings, 17, 421 (1958).

3) y-3-Pyridyl-y-methylaminobutyric acid as a urinary metabolite of nicotine, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 79, 6342 (1957).

4) Metabolites of nicotine and a synthesis of nornicotine, Herbert McKennis, Jr., Lennox B. Turnbull, Harvey N. Wingfield, Jr. and Lovell J. Dewey, Journal of the American Chemical Society, 80, 1034 (1958).

5) The role of cotinine in nicotine metabolism, Herbert McKennis, Jr., L. B. Turnbull and E. R. Bowman, Abstracts of Communications, 14-37, IV International Congress of Biochemistry, Wien 1-6 September 1958.

6) A constant rate infusion apparatus, Quentin S. McKennis, Edward R. Bowman and Herbert McKennis, Jr., Toxicology and Applied Pharmacology, I, (1959) (in press).

7) Metabolism of nicotine to y-(3-pyridyl)-y-methylaminobutyric acid, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 80, (1958) (in press).

8) Metabolism of nicotine in the human and excretion of pyridine compounds by smokers, Edward R. Bowman, Lennox B. Turnbull and Herbert McKennis, Jr., Science (submitted for publication, November 26, 1958).

Four additional manuscripts have been partially completed.